Histochemical study of the skin affected by certain autoimmune diseases

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Abstract

This study puts forth the morphological and metabolic modifications that occurred in the skin affected by *Pemphigus vulgaris*, modifications that have been studied by histoenzymatic techniques. There were studied skin biopsies from patients suffering from *Pemphigus vulgaris*, hospitalized in Dermatology services in Bucharest. The pieces were sectioned at cryotome, at 3^0C and then they were studied with histochemical methods, in order to evidentiate the activity of the following enzymes: $NADH_2$-cytochrome-c-reductase, lactate-dehydrogenase (LDH), dihydrofolic-reductase (DHFR), folic acid (FA), ATP-ase pH 9.4, leucin-amino-peptidase (LAP). The enzymatic activity was appreciated in all skin components: epidermal layers, connective tissue cells and inflammatory cells from the superficial and deep dermis, blood vessels, nerve fibers and encapsulated corpuscles. In the affected skin, there were observed modifications of all enzymes studied. The study revealed the following aspects: the balance between oxybiotic and anoxybiotic metabolism in the epidermal cells changes from the predominance of the former in the healthy skin, to the dominance of the latter in the affected skin, an active turn-over of the nucleic acids and increased synthesis of amino acids in the dermal cell population (mainly in the fibroblasts and mast cells), morphological and functional alteration of mitochondria, lysosomes and cellular membranes.

***Keywords*:** skin, *Pemphigus vulgaris*, histochemistry.

Introduction

The skin and its epithelial and interstitial structures offer an important field of study, and the skin diseases are regarded today from a new point of view. The scientific studies of intracellular processes, immune and metabolic (enzymatic) processes have in depth the knowledge about integument diseases.

All these were possible due to modern technology of investigation: histochemistry, histoenzymology, electron microscopy, imunohistochemistry that brought new data also in dermatology. Thus, new data have occurred about multi-potentiality of fibroblasts, repair mechanism in the dermis based on fibroblastic activity either singly, or associated with local mast cells, macrophages and migratory cells from the blood stream [1–3].

This study puts forth the morphological and metabolic modifications that occurred in the skin affected by *Pemphigus vulgaris*, modifications that have been studied by histoenzymatic techniques.

Material and methods

There were studied 23 skin biopsies from patients suffering from *Pemphigus vulgaris*, hospitalized in Dermatology services in Bucharest. The pieces were sectioned at cryotome, at 3^0C and then they were studied with histochemical methods, in order to evidentiate the activity of the following enzymes:

– $NADH_2$-cytochrome-c-reductase.
– Lactate-dehydrogenase (LDH).
– Dihydrofolate-reductase (DHFR).
– Folic acid (FA).
– ATP-ase pH 9.4.
– Leucin-amino-peptidase (LAP).

Results and discussions

$NADH_2$-cytochrome-c-reductase

In the epidermis, the enzymatic activity is medium and intense positive in the basal layer, weakly positive in the prickle layer and negative in all superficial layers. In the papillary dermis are present numerous groups of inflammatory cells, higher number than in the reticular dermis. In these cells, macrophages respectively, the activity of $NADH_2$-cytochrome-c-reductase has different enzymatic levels: intense and very intense activity predominates.

Macrophages from the reticular dermis show only medium and intense activity of the enzyme. Plasma cells have very intense enzymatic activity. Polymorphonuclears and mast cells show an intense activity for this enzyme. Fibroblasts are displaced by inflammatory infiltrate and show weak, medium and intense enzymatic activity (Figure 1).

In the reticular dermis, connective tissue cells are located along the collagen fibers, elongated in shape and having an intense or very intense enzymatic activity. Mast cells, plasma cells and macrophages are intense positive while neutrophils and lymph cells show a medium activity for the enzyme. Blood vessels, both arterioles and venules, show intense activity for the enzyme in the endothelial cells of the tunica intima and

in the smooth muscle cells from the tunica media. Myelinated nerves posses an intense enzymatic activity.

The sensory corpuscles may show weak enzymatic activity (Meissner's corpuscles), or have different levels: very weak or very intense (Vater Pacini's and Ruffini's corpuscles).

Lactate-dehydrogenase (LDH)

In the epidermis, the cells of the basal and prickle layer display an intense activity for LDH. The cells in the upper layers, as in the granular layer display a medium level of the enzymatic activity. The superficial layer is negative for this enzyme. In the dermis, the cells in inflammatory infiltrate have different levels of enzymatic activity: very weak, weak, medium, intense and very intense reactions.

These different reactions are present both in the superficial and deep dermis for all cell types: macrophages, lymph cells, and plasma cells. The most active are macrophages. Mast cells display medium levels for lactate-dehydrogenase activity. Most of them are grouped close to the epidermal-dermal junction and are only sparse in the reticular dermis (Figure 2).

The fibroblasts from the superficial and deep dermis show different levels of LDH activity. The blood vessels have strong enzymatic reactions in the endothelium and variable LDH responses in the media. Myelinated fibers have a medium enzymatic response for LDH, while the encapsulated corpuscles show a weak enzymatic activity.

Dihydrofolate-reductase (DHFR)

In the epidermis, the enzymatic activity is very weak in the basal layer and is negative in the superficial layers. In the dermis, the fibroblasts show different levels of dihydrofolic-reductase activity, which progressively increases in reticular dermis.

The cells in inflammatory infiltrates: lymphocytes, plasma cells, macrophages and plymorphonuclears vary in enzymatic activity from very weak to very intense. Mast cells attached to the basement membrane display intense enzymatic activity (Figure 3).

The blood vessels of all types show medium levels of activity in the endothelial cells and in the smooth muscle cells of the tunica media. Myelinated nerves have medium or intense enzymatic levels and encapsulated corpuscles show a medium reaction for dihydrofolic-reductase.

Folic acid

In the epidermis: part of basal and prickle cells posses a weak reaction for folic acid. In the dermis: fibroblasts show reactivity that range from medium to intense values. Some cells show enzymatic reaction in the cytoplasm, some in the nucleus, and others in both nucleus and cytoplasm (Figure 4).

In inflammatory infiltrates, cells as lymphocytes, plasma cells, macrophages, eosinophils show intense reaction for folic acid. Mast cells display medium and intense reaction. Blood vessels display medium and intense enzymatic activity in endothelial cells and in the arterial tunica media.

Myelinated nerves and encapsulated corpuscles show a medium and intense enzymatic activity for folic acid.

ATP-ase pH 9.4

In the epidermis the enzymatic activity is weak in the basal cells, while in the prickle cells the reaction ranges from weak to medium. In the dermis, blood vessels in the papillary and reticular dermis show intense endothelial enzymatic reaction. ATP-ase activity is very intense in macrophages located around the blood capillaries (Figure 5).

Inflammatory infiltrates occupy large areas in the dermis and they show a variable intensity of enzymatic activity: weak, medium and intense. Fibroblasts in the superficial dermis show weak ATP-ase activity, while those in the deep dermis are intensely reactive.

Mast cells are seldom seen and show weak reaction for ATP-ase. Branched melanocytes occur among basal cells and they display a very intense enzymatic activity. Nerve fibers show medium and intense ATP-ase levels in Schwann cells. The blood vessels in the epineurium are intensely positive.

Leucine-amino-peptidase

In the epidermis is present a very weak reaction of the basal cells. In the other epidermal layers the reaction is negative. The cellular components of the papillary dermis (lymphocytes, plasma cells, eosinophils and mast cells) display intense cytoplasmic enzymatic reactions. Macrophages are intensely positive. Fibroblasts show enzymatic reactions of different levels, but those with medium levels of enzymatic activity predominate (Figure 6).

In the reticular dermis, the reactive cells are seldom seen and they show a weak enzymatic reaction. Fibroblasts and mast cells have a moderate reactivity for leucin-amino-peptidase, while macrophages are intense positive for this enzyme.

The blood vessels from superficial dermis display intense enzymatic activity, those in the deep dermis have a medium level of leucin-amino-peptidase activity in the cytoplasm of the endothelial cells. Myelinated nerves display moderate and intense reaction in Schwann cells and in the concentric layers of the sensory corpuscles.

There were observed modifications of all enzymes studied. Weak activity of the $NADH_2$-cytochrome-c-reductase proves an important decrease of oxybiotic glycolisis, while the intense and very intense activity of lactate-dehydrogenase shows an increased anoxybiotic glycolisis [4, 5].

Cellular organelles, especially mitochondria, the site of $NADH_2$-cytochrome-c-reductase activity, are affected.

The activity of lactate-dehydrogenase, the cytosolic, glycolitic anoxybiotic enzyme is increased and occupies a central role. The ratio between the two enzymes changes: in the healthy skin oxybiotic glycolisis predominates, while in the skin affected by *Pemphigus vulgaris*, the anoxybiotic glycolysis is the main metabolism type.

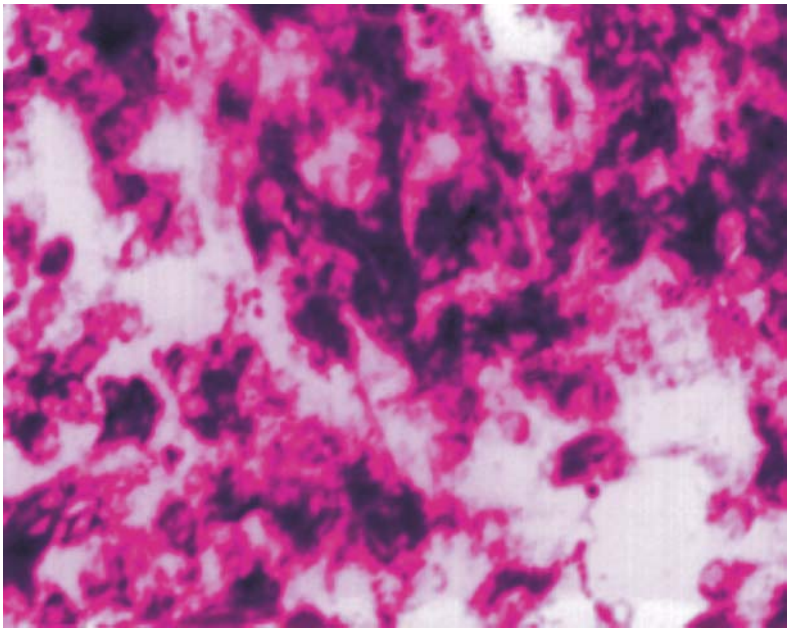

Figure 1 – *$NADH_2$-cytochrome-c-reductase. Papillary dermis with connective tissue cells and inflammatory cells that display different levels of enzymatic activity. Intense and very intense reactions predominate*

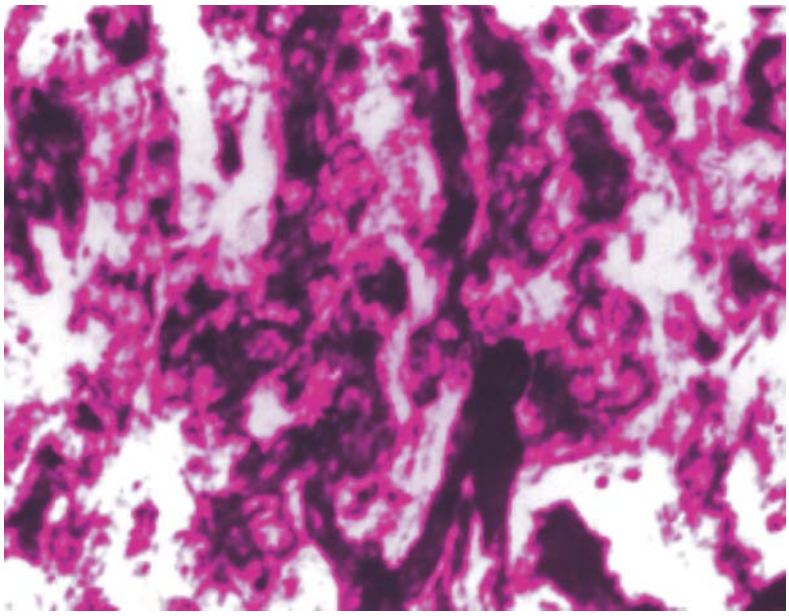

Figure 2 – *Lactic-dehydrogenase. Cell-rich papillary dermis with intense and very intense cytoplasmic reactions. Many macrophages are intensely positive. Blood vessels wall has intense enzymatic levels*

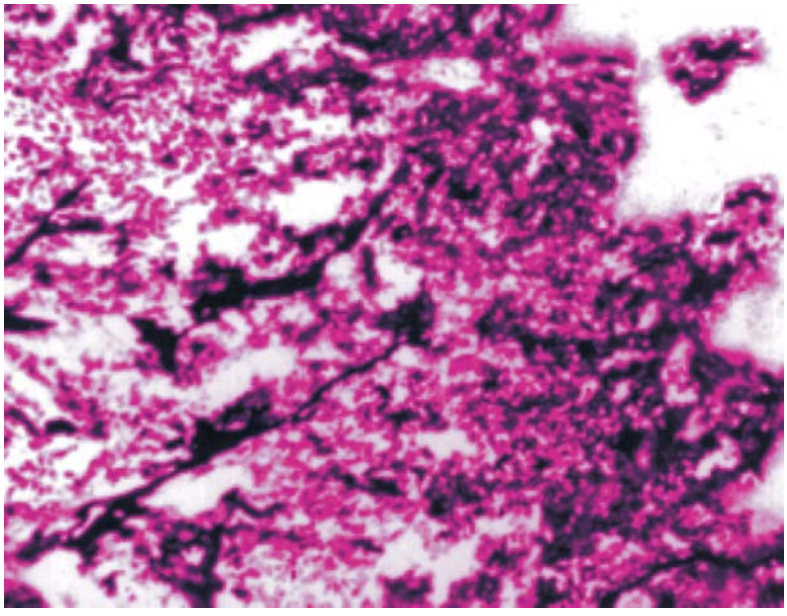

Figure 3 – *Dihydrofolic-reductase. Epidermis displays no enzymatic activity. The connective tissue and inflammatory cells, as the vascular component of the superficial dermis, are all positive for DHFR activity*

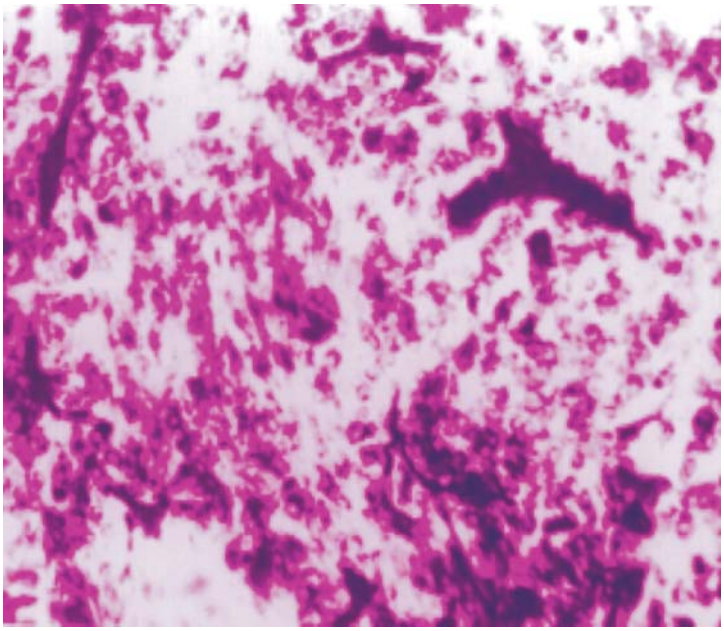

Figure 4 – ***Folic acid. Papillary dermis with many macrophages with very intense enzymatic activity and intense positive blood vessels***

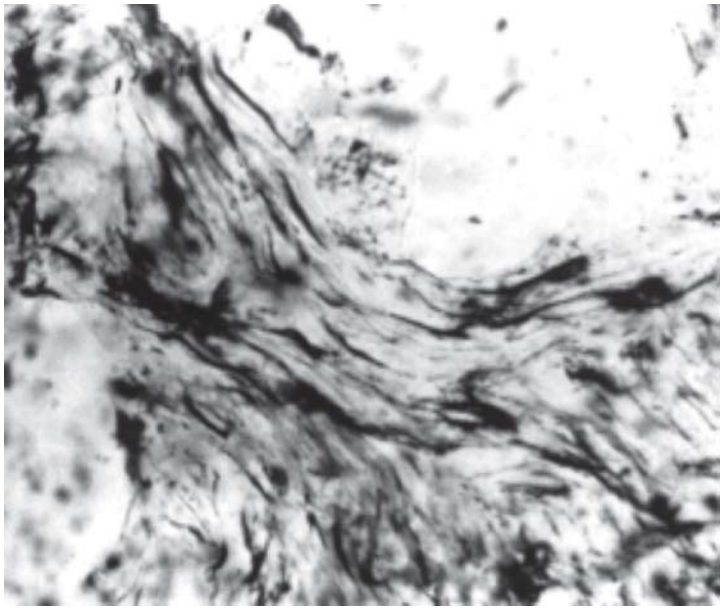

Figure 5 – ***ATP-ase pH 9.4. Superficial dermis from the vicinity of two pustules. There are present blood vessels, most of them capillaries with very intense enzymatic reaction***

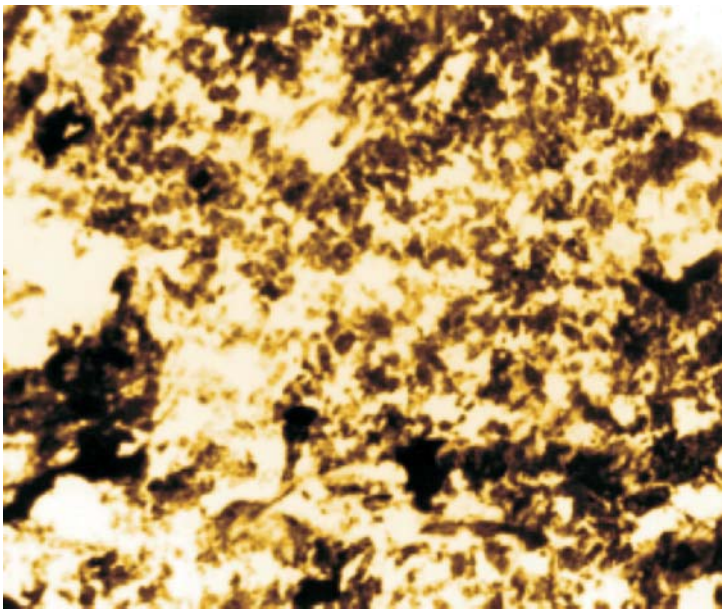

Figure 6 – ***Leucin-amino-peptidase. Important inflammatory infiltrate in the superficial dermis. Most of the inflammatory cells are intense and very intense positive for LAP activity***

Folic acid has reduced activity, which results in a decreased folic metabolism. Thus, the turnover of nucleic acids and the synthesis of certain amino acids (serine, glycine) are altered [6–8].

The activity of dihydrofolate-reductase, the key enzyme for folic acid metabolism is also affected, because mitochondria are morphologically and functionally modified [9].

In turn, the ATP-ase pH 9.4, together with lactate-dehydrogenase, has an increased level of activity. ATP-ase pH 9.4 is a membrane enzyme. Thus, the plasma membrane and the endomembranes are modified in *Pemphigus vulgaris* [10].

Membrane-bounded organelles are seriously altered. It is presumed that the affected endomembranes modify the function and the activity of these organelles. Increased levels of ATP-ase pH 9.4 prove abnormal, pathologic changes between organelles and surrounding cytosol, rather than improved functioning.

Leucin-amino-peptidase, localized on lysosomes has also decreased levels of its activity during illness. This results into a weaker defense reaction because of acid hydrolases deficit [11].

The lysosomes may be partially or totally disintegrated [12–20].

Conclusions

The oxidative metabolism is extremely decreased in the epidermis. The oxybiotic metabolism is present only in the cells of the basal layer. Anoxybiotic metabolism predominates and is present prickle layer and even in the granular layer of the epidermis. The metabolism of folic acid derivatives is weak in the epidermis, but very intense in the papillary and reticular dermis, in all cell types. This proves an active turnover of the nucleic acids and an increased synthesis of certain amino acids, like glycine and serine in which folic acid and its enzyme are directly involved.

The activity of leucine-amino-peptidase is enhanced in all dermal cell types, particularly in lysosomes – rich cells that develop phagocytic activities (macrophages, neutrophils, eosinophils, endothelial cells).

There is a sharp metabolic difference between epidermis and dermis and also between the components of papillary and reticular dermis.

In the dermis of the skin, the metabolic activities are enhanced, but there are differences between the enzymatic reactions in fibroblasts and macrophages, less significant between lymphocytes and plasma cells and similar for blood vessels and nerves.

In the deep dermis predominates the synthesis of new connective tissue components (fibers, matrix proteins). This means the fibroblasts (fiber synthesis, glycosaminoglycans and proteoglycans production) and mast cells (matrix protein synthesis) have the main metabolic role.

The two cell types show intense enzymatic reactions. Both in papillary and reticular dermis, there are different metabolic alterations of connective tissue cells: some cells display different enzymatic levels (fibroblasts) and in another category of cells the enzymatic reactions differ according to the illness phase: acute, subacute, chronic (plasma cells, mast cells, eosinophils, lymphocytes).

The blood vessels of the dermis display intense enzymatic activity. This proves that blood vessels participate, together with all cell types (fixed and wandering) to maintain a normal metabolic rate of the skin, in the attempt to cease the pathologic process.

Based on these observations we came to the conclusion that the skin affected by *Pemphigus vulgaris* develops active processes of: fiber synthesis (fibroblasts), phagocytosis and local defense response (macrophages), glycosaminoglycan and proteoglycan molecules renewal (mast cells) and immune response (lymphocytes, plasma cells, macrophages).

Myelinated nerves show intense enzymatic activity for all enzymes, except for lactate-dehydrogenase which has a weak reactivity. The most active nerve structures are Schwann cells with important role in nerve impulse conductivity, nerve regeneration and local metabolism.

Encapsulated nerve endings have a diverse metabolic reaction. The superficial Meissner's corpuscles are more affected than the deep Vater-Pacini's and Ruffini's corpuscles which show higher levels of enzymatic activity.

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